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"CONTACT REACTIONS" BETWEEN XENOGENEIC OR ALLOGENEIC COELOMIC CELLS OF SOLITARY ASCIDIANS

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The ability to distinguish self and nonself components seems to be widespread throughout the animal kingdom (Hildemann and Reddy, 1973; Cooper, 1976). The vertebrates, from the cyclostomes onward, are capable of mounting specific humoral as well as cell-mediated immune responses in defence against invading nonself components. In invertebrates, however, it is only in advanced organisms that recognition of foreignness is followed by selective destruction or elimination of such material, and even then this is accomplished only by means of cell-mediated mechanisms. It is as yet unclear whether invertebrates possess an ability for "adaptive immunity" as defined by the presence of specific anamnesis, even though primitive forms of "memory" have been demonstrated in certain annelid worms (Cooper, 1969). It would seem likely, therefore, that a sharp gap exists between vertebrates and invertebrates in the evolutionary stages attained by their defence mechanisms. However, evolution of the mechanisms by which self and nonself cell-surface components are recognized in the animals is itself of considerable interest, quite apart from the origin of active rejection mechanisms subsequent to nonself recognition. The importance of such self–nonself recognition beyond its role in self-defence is underlined by the fact that the genes of the major histocompatibility complexes of higher vertebrates are known to control physiological interactions between syngeneic cells (Katz, 1977).

Ascidians, a group of urochordates, occupy a unique position in the metazoan phylogeny, as these animals are often regarded as related to the immediate forerunners of the vertebrates. Studies of self–nonself recognition in ascidians are therefore particularly relevant, since such studies might reveal crucial evolutionary steps towards the sophisticated cell-interaction systems of vertebrates. From this point of view, extensive studies of the colonial ascidians have been particularly rewarding. Using *Botryllus primigenus,* Oka and his colleagues have shown that colonies which share an allele of the single "histocompatibility" locus fuse with each other, whereas colonies lacking a shared allele exhibit mutual non-fusion reactions (Oka and Watanabe, 1957; Oka, 1970; Tanaka and Watanabe, 1973). The genetic control of allogeneic reactivity in this system thus follows a rule which clearly differs from the familiar rules of transplantation genetics established for the vertebrates. Nevertheless, these animals must possess genetically specific recognitive ability for self components, coded for or controlled by the "histocompatibility" locus. Moreover, a strict correlation was found between alloreactivity and the ability of gametes from two colonies to fertilize each other, raising an intriguing possibility that the "histocompatibility" locus in the ascidians may control cell-to-cell interactions in general (Oka and Watanabe, 1957; Oka, 1970).

Solitary or simple ascidians have so far received much less attention than the colonial or compound ascidians. In a previous paper, we reported the nature of naturally occurring hemagglutinins in the coelomic fluid of such ascidians. The

substance(s) appeared to be a mucopolysaccharide unrelated to immunoglobulins (Fuke and Sugai, 1972). I now report studies on a nonphagocytic cellular reaction of the solitary ascidians against xenogeneic and allogeneic cells. When isolated coelomic cells from two species of solitary ascidians were brought into contact *in vitro*, both cells reacted against each other, resulting in reciprocal lysis of both cells. Moreover, coelomic cells of one of such species, *Halocynthia roretzi* (Drasche), were studied for their allogeneic reactivity and were found to exhibit the same type of cellular reactions in some but not all combinations of different individuals. This cellular reaction, henceforth called "contact reaction," will be described in detail below. The reaction can be regarded as a cellular counterpart of nonfusion reactions in the colonial ascidians.

Materials and Methods

The following six species of solitary ascidians were employed: *Halocynthia roretzi* (Drasche), *H. aurantium* (Pallas), *Pyura mirabilis* (Drasche), *Styela clava* (Herdman), *Ciona intestinalis* (Linnaeus), and *C. robusta* (Hoshino and Tokioka). *H. aurantium* specimens were obtained at Otaru, Hokkaido; the rest were collected in Mutsu Bay, Aomori. All experiments were carried out at the aquarium facilities of the Marine Biological Station, Asamushi, Aomori. Three variants of *H. roretzi*, designated as Types A through C, have been described in detail (Numakunai and Hoshino, 1973).

Coelomic cells of *H. roretzi, H. aurantium,* and *P. mirabilis* were collected from the mantle-test interspace by withdrawing body fluid into hypodermic syringes containing a five- to ten-fold excess of sea water. Those from *S. clava* and the two species of *Ciona* were obtained through incisions made in the test and the mantle. Up to 5×10^6 coelomic cells of each animal to be tested were cultured in glass chambers measuring $15 \times 25 \times 1$ mm deep. Unless otherwise stated, xenogeneic or allogeneic reactions were observed as follows: Initially, the coelomic cells from one animal were cultured for 5 min. The culture fluid was discarded and the cells which adhered to glass surfaces were washed several times with fresh sea water before a suspension of cells from a second animal was introduced into the chamber. The chamber was placed under a phase-contrast microscope and the reactions were observed at a magnification of 1000 ×. Normally, cells from different species were readily distinguished by morphological criteria (Fuke, 1979). Cells from the two *Ciona* species, however, were morphologically alike and had to be distinguished from each other by following, under an inverted microscope, the settlement of the cells from the second animal on the adhered cells from the first animal. In experiments involving cells from conspecific variants or individuals, vital staining of cells from one animal served to identify the donor of each cell. The vital stain employed was either Toluidin Blue or Nile Blue, both at 0.001% concentration. The viability and reactivity of stained cells were indistinguishable from those of unstained cells.

Since sperm of one individual could be freed of autologous eggs, but not vice versa, fertilization experiments were carried out by mixing separated spermatozoa from one animal with an egg-sperm mixture from another animal.

Results

The phenomenon of contact reaction and the cell types involved

When coelomic cells of one animal were brought into contact *in vitro* with such cells from a different animal (except for certain allogeneic combinations; see

below), a series of cellular events, here termed "contact reaction," ensued. The reaction can be followed by time-lapse photographs: In the first example, a reaction between xenogeneic cells was recorded (Fig. 1). Coelomic cells of *H. roretzi* were cultured for 5 min and washed once with sea water. Cells of *P. mirabilis* were then introduced to the culture chamber. Shortly after the two xenogeneic vesicular cells came into contact (Fig. 1a), the *H. roretzi* cell started moving around the *P. mirabilis* cell, which remained stationary (Figs. 1b and 1c). The former then ceased movement and appeared to press itself tightly to the latter (Fig. 1d). Within seconds, the *H. roretzi* cell was lysed (Fig. 1e), followed immediately by lysis of the *P. mirabilis* cell (Fig. 1f). In this particular pair of cells, the *H. roretzi* cell appeared to initiate the whole process of the contact reaction by assuming the role of an "attacker" cell. However, in many other similar pairs of cells in the same culture, a *P. mirabilis* cell acted as an apparent "attacker." Not infrequently, moreover, two cells in a pair took turns moving around each other, indicating that the two cells in a reacting bicellular conjugate are "attackers" and "targets" at the same time. It was unpredictable which of the two cells in contact would be lysed first, even when the apparent "attacker" could be discerned from the "target." In any case, lysis of one cell was always followed immediately by that of the other cell. In this reaction, therefore, distinction between "attacking" cells and "target" cells is superfluous because of their complete reciprocity. This sharply contrasts the ascidian contact reaction from cell-mediated cytotoxicity in higher animals. Moreover, pairs of cells sometimes proceeded to the "tight adherence" stage without undergoing the initial "maneuver" of one of the cells around the other, suggesting that such preparatory movement is not a prerequisite for subsequent reactions.

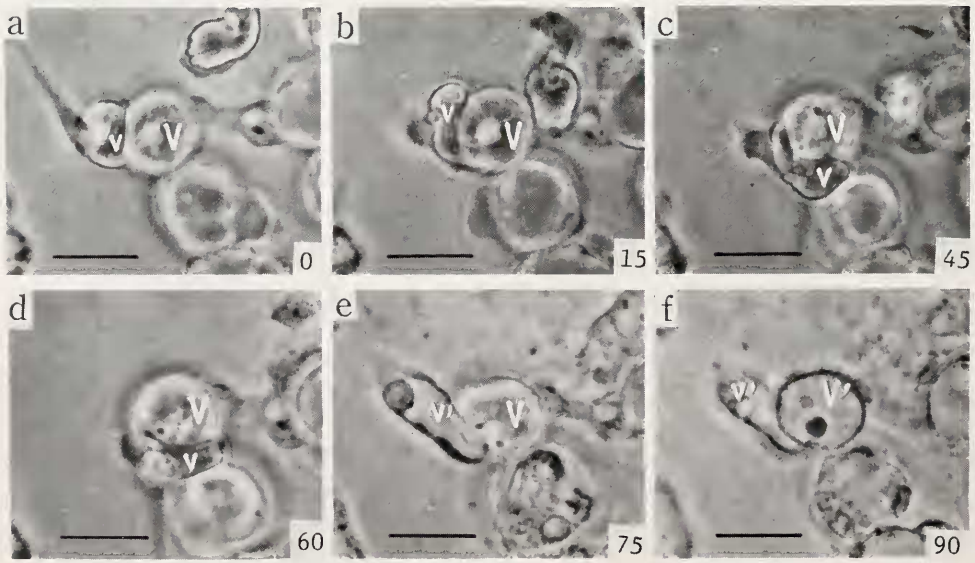

FIGURE 1. Contact reaction between vesicular cells of *P. mirabilis* (V) and *H. roretzi* (v). Upon contact (a), the v cell moves around the V cell (b, c). The movement stops and the v cell presses itself to the V cell (d). Th v cell is then lysed (v', in e), followed promptly by the lysis of the V cell (V', in f). Scale bar = 10 μ. The time required for the reaction in seconds is shown at the lower right corner of each picture.

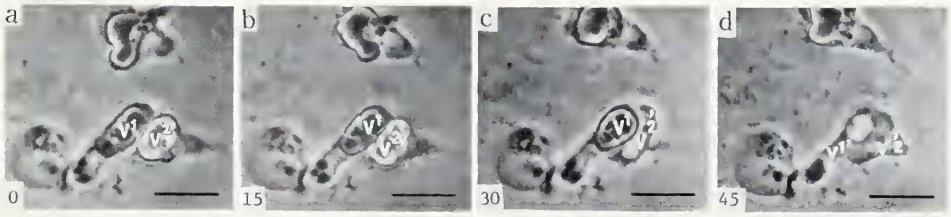

FIGURE 2. Contact reaction between vesicular cells (v^1 and v^2) from two individuals of Type C *H. roretzi*. One cell, v^1 was vitally stained with Nile Blue and appears darker than the second, unstained, v^2 cell. Following contact (a), both cells remain stationary (b), followed by lysis of v^2 ($v^{2\prime}$, in c) and then v^1 cell ($v^{1\prime}$ in d). Scale bar = 10 μ. Time elapsed after the cell contact is indicated in seconds at the lower left corner of each frame.

Xenogeneic contact reactions were observed in congeneric species pairs as well as between species of different genera, for example, *H. roretzi* vs. *H. aurantium*, *H. roretzi* vs. *P. mirabilis*, *H. roretzi* vs. *S. clava*, *P. mirabilis* vs. *S. clava*, and *C. intestinalis* vs. *C. robusta*. The strength of contact reactions in various species pairs, judged by the frequency of reactions and the average time required from cell contact to lysis, was roughly the same except possibly for reactions involving the two *Ciona* species, whose reaction seemed somewhat weaker than that between other xenogeneic combinations examined.

Three variants of *H. roretzi*, designated as Types A, B, and C, have been described by Numakunai and Hoshino (1973). They differ in peak daily spawning hours, reproductive season, and preferred habitat, as well as in external morphology. Cells from different variants, when tested by mixing *in vitro*, exhibited vigorous contact reactions in all combinations of the three types. Although these three variants are taxonomically considered conspecific, genetic differences between any two types are probably intermediate between xenogeneic and allogeneic. It was therefore of interest to test whether cells from different individuals within the same variant could react with each other. In such experiments, contact reactions were observed in most but not all pairs, as discussed below. When an allogeneic contact reaction did occur, however, it was in all respects indistinguishable from xenogeneic reactions. Figure 2 shows an example of allogeneic contact reaction between vesicular cells from two Type C *H. roretzi* individuals. As in xenogenic reactions, the cells, upon establishing contact, adhered tightly to each other (Figs. 2a and 2b). This was followed by lysis of one of the cells (Fig. 2c) and then of the other (Fig. 2d).

The exact nature of "lysis" in these reactions and the fate of "lysed" cells are unclear. Since most of the coelomic cell types involved in this reaction (see below) contain vesicles of various sizes, the "lytic" process is readily detected as discharge of such vesicles from the cells undergoing contact reactions. The vesicles at the same time disintegrate and release their contents into the surrounding medium. A "lysed" cell thus shows vacuoles, and remains totally immobilized for at least several hours while still in tight contact with the other, also "lysed," cell. Following such a motionless period, at least some of the "lysed" cells extended their pseudopodia and apparently loosened themselves from the partners, suggesting that the contact reaction might not necessarily entail cell death.

Contact reactions were observed between cells of the same morphological type as well as between cells of different types. Those which participate in contact reactions are vesicular cells, large granular amoeboid cells, small granular amoe-

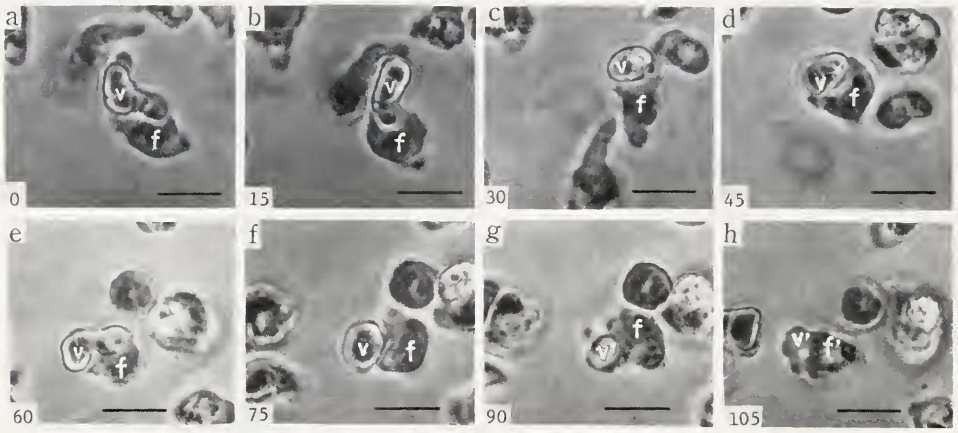

FIGURE 3. Contact reaction between a fine-granular amoeboid cell (f) and a vesicular cell (v) from two Type C *H. roretzi* donors. The f cell has been stained with Nile Blue and is shown darker than the unstained v cell. The f cell moves around the v cell (a–f), until the latter discharges vesicles (v', in g). The f cell itself is also lysed soon afterwards (f', in h). The scale bar indicates 10 μ. The time after initiation at which each picture was taken is shown in seconds in lower left corners.

boid cells, minute granular cells, fine granular amoeboid cells, large basophilic cells, and the so-called vacuolated cells that differ from "lysed" cells of various types (for nomenclature and identification of various cell types, see Fuke, 1979). Whether other cell types, such as brown cells, orange cells, and "lymphocytes," also engage in contact reactions has not been determined because of their low frequencies among coelomic cells. An allogeneic contact reaction between a fine-granular amoeboid cell and a vesicular cell is recorded in Figure 3. The former cell initially moved around the latter and the lysis of the latter cell can be seen by its vesicular discharge (Fig. 3d).

Requirement for direct cell contact

Spontaneous lysis of coelomic cells was never observed in cultures of cells from a single animal, regardless of whether or not the cells were in contact with another autologous cell. Hence, autologous contact reactions do not occur. In mixed cultures of nonautologous cells, except for certain combinations of allogeneic cells, cytolysis was apparently triggered only when two heterologous cells came into direct contact. However, under the experimental conditions employed, most of the mixed cultures contained some coelomic fluid carried over from one or the other cell donor. It seemed conceivable, therefore, that the presence of heterologous body fluid made cells more susceptible to spontanous lysis and that, if so, at least some of the cells might be lysed without direct cell contact. Once such spontaneous cytolysis did occur, it could possibly lead to nonspecific lysis of proximate cells (see below). Effect of heterologous coelomic fluid on spontaneous lysis was therefore tested. Additionally, culture media in which vigorous contact reactions had previously taken place were also tested to see if incidence of spontaneous lysis could be increased when fresh cells were deliberately exposed to such media.

Cell-free coelomic fluid from *H. roretzi* or *P. mirabilis* was obtained by centrifugation. Cells from one or the other species were cultured in glass chambers, washed several times with sea water and then cultured with coelomic fluid from either species. Regardless of the source of fluid, cells of these two species were perfectly normal during several hours' observation and showed no indication of spontaneous lysis.

Coelomic cells from *H. roretzi* and *P. mirabilis* were cocultured for 30 min, a period long enough to ensure completion of contact reactions, and the culture medium (*i.e.*, mixed coelomic fluid) was collected. Fresh *H. roretzi* or *P. mirabilis* cells then were cultured separately in the medium containing material released from lysed cells. Cells from either animal in such cultures behaved exactly like the cells in control cultures for which fresh autologous coelomic fluid was used. Similar experiments also were carried out with cells from two alloreactive individuals using media in which allogeneic contact reactions took place, and the results were the same.

These experiments confirm the absence of spontaneous lysis and autologous contact reactions in xenogeneic or allogeneic mixed cultures. In addition, they strongly suggested that a contact reaction is a specific reaction triggered by direct cell contact between xenogeneic or allogeneic cells. However, in contrast to the requirement of direct cell contact in triggering contact reactions, effector mechanisms of these reactions may not necessarily require cell contact. When contact reactions between vesicular cells of *H. roretzi* and *P. mirabilis* took place, some of the nearby *P. mirabilis* vesicular cells were found to be lysed without prior contact with *H. roretzi* cells. In fact, lysis of one cell appeared to trigger a chain reaction among nearby vesicular cells. Since supernatant from cultures in which contact reactions had taken place did not have such an effect, the factor(s) which caused this chain reaction either was labile or was effective only over a short range. Vesicular cells of *P. mirabilis* contain particularly large vesicles and they tended to be more sensitive to routine manipulations than other cells, perhaps accounting for the observation of such spontaneous lysis only in this cell type. Whether or not this short-range reaction in the absence of cell contact is analogous to the lysis initiated by cell contact remains to be elucidated. It seems possible, however, that lysis of the second cell in a cell pair undergoing contact reaction is a direct consequence of lysis of the first cell, the two cells being as close to each other as they can. This observation indicates that cytolysis can be mediated by diffusible material, probably derived from vesicles. Nevertheless, it should be emphasized that lysis of the first cell in a bicellular conjugate is in all likelihood effected by direct cellular interactions.

Finally, despite the lack of autologous contact reaction between healthy cells, it is conceivable that degenerated cells are eliminated by autologous cells via a similar reaction. To test this, fresh *H. roretzi* cells were mixed with autologous cells that had been cultured for up to 12 hr. Even under such circumstances, no autologous reactions ensued, presumably because such reactions do not play a role in removing aged cells, or the culture period was too short to produce cells sufficiently degenerated to elicit autologous reactions.

Contact reaction in vivo

The above observations are concerned only with contact reactions in an artificial, *in vitro,* system. An obvious question is whether the same type of reaction

TABLE I

Reciprocal contact reactivities among 16 Type A individuals of H. roretzi. *Plus (+) and minus (−) denote presence and absence of contact reactions, respectively.*

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	No. 16
No. 1	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
No. 2	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+
No. 3	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
No. 4	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
No. 5	+	+	+	+	−	+	−	+	+	+	+	+	+	+	−	+
No. 6	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+
No. 7	+	+	+	+	−	+	−	+	+	+	+	−	+	+	−	+
No. 8	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
No. 9	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+
No. 10	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+
No. 11	+	+	+	+	+	+	+	+	+	−	−	+	+	−	+	+
No. 12	+	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+
No. 13	−	+	+	+	+	+	+	+	+	−	+	+	−	−	+	−
No. 14	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+
No. 15	+	+	+	+	−	+	−	+	−	+	+	+	+	+	−	+
No. 16	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−

can be demonstrated in intact animals. To answer this question, coelomic cells of *P. mirabilis* were marked with a vital stain and their suspensions in a volume of 0.4 ml were injected between mantle and test inside the papillae of *H. roretzi.* Twenty min later, coelomic fluid from the site was withdrawn with a syringe and the cells were examined microscopically. None of the donor type, *i.e.,* stained, cells were single, all being surrounded by one or more unstained, host-derived cells. About $\frac{2}{3}$ of the donor cells had vacuoles, indicating vesicle discharge following contact reactions. The rest of the cells had normal vesicles, *i.e.,* had not been lysed. Therefore, the timing and features of *in vivo* reactions were quite similar to the observations made *in vitro,* strongly indicating that a contact reaction can be induced *in vivo* as well as *in vitro.*

Pattern of allorcactivity in H. roretzi

As already pointed out, cells from different individuals of the same variant of *H. roretzi* exhibited contact reaction in most but not all combinations of individuals. In a preliminary experiment, for instance, cells from one Type C individual were tested against those from six other Type C animals. One of the six failed to react with the first individual. In another experiment with the Type C animals, cells from one individual did not react with those from one out of nine individuals tested. Thus, cells from at least some individuals behaved as if they were autologous. However, two individuals which do not react wth each other are not necessarily identical, since they might react differently with a third individual. It was, therefore, of considerable importance to analyse patterns of reciprocal reactivity by using a large panel of individuals.

Sixteen Type A individuals were collected from an area of approximately 10 m² near Futago-jima Island in Mutsu Bay, and were subjected to mixed culture experiments in all possible combinations. The results of this experiment, involving a total of 120 combinations, are shown in Table I. Three individuals

(Nos. 3, 4, and 8) reacted with all others, whereas one animal (No. 13) failed to react with as many as four other individuals. Thus, the frequency of non-reactive allogeneic combinations for each individual ranged from 0/15 to 4/15. All except two of the 16 animals were different in their alloreactivity patterns. For instance, animals No. 5 and 7 did not react with each other and showed parallel reactivity towards Nos. 9 and 15, yet differed in reactivity against animal No. 12. Two exceptions were Nos. 10 and 14. They did not react with each other and were identical in being reactive with all others except for Nos. 11 and 13. Thus, these two behaved as if they were "syngeneic" in histocompatibility as far as this test was concerned. Obviously, however, these two might have reacted differently had another individual been added to the test panel.

Lack of correlation between alloreactivity and fertilization

Induction of a contact reaction requires direct contact between cells, and hence it involves mutual recognition of cell surface components. A vital, naturally occurring process which depends on such cellular recognition is fertilization. In the solitary ascidians studied here, each individual simultaneously releases sperm and eggs through the atrial aperture. However, self-fertilization, *i.e.*, fertilization between autologous spermatozoa and eggs, is known to occur, usually at a low rate (1–5%). In contrast, the frequency of fertilization between gametes from different individuals is as high as 80 to 90%. This well-known self sterility raises an interesting question, namely whether there is a correlation between alloreactivity and the frequency of fertilization. In a colonial ascidian *Botryllus primigenus*, Oka and Watanabe (1967) found a strict correlation between fertilization and "non-fusion" reaction, an expression of alloreactivity which in many respects resembles contact reactions of solitary ascidians described in the present study. In the colonial ascidians, fertilization occurred only between alloreactive colonies, suggesting that cell surface components involved in fertilization and alloreactivity are under the same genetic control.

To test if the rules observed in the colonial ascidians also apply to solitary ascidians, the frequency of fertilization between pairs which are either positive or negative in contact reactivity was examined. In the first experiment, the frequency of fertilization between two individuals that showed contact reaction (Nos. 8 and 9 in Table I) was found to be about 94% and the majority of fertilized eggs developed into tadpoles (Experiment 1, Table II). In subsequent experiments, however, similar high frequencies of fertilization were obtained even between individuals which did not exhibit contact reactions, for instance between animals Nos. 10 and 11, or between Nos. 13 and 16, irrespective of which one of the pair provided the egg or the sperm (Experiments 2 and 3, Table II). These results therefore indicate that the alloreactivity in terms of contact reactions is irrelevant for fertilization.

Discussion

Ascidians are capable of recognizing foreign material and of eliminating it from their bodies. In a solitary ascidian, *Molgula manhattensis*, hemocytes either encapsulate or phagocytize such material for removal (Anderson, 1971). Coelomic cells of solitary ascidians phagocytize and eliminate sea urchin spermatozoa and fixed mammalian erythrocytes (Fuke, 1979). Observations reported in the pres-

TABLE II

Lack of correlation between contact reactivity and fertilization in H. roretzi *Type A individuals. The animals tested were some of those employed in the experiments shown in Table I, and are assigned the same numbers.*

	Contact reaction of coelomic cells	Developmental stage 48 hr after mixing (% of total scored)		
		Uncleaved egg	Cleavage stage, blastula, gastrula, or neurula	Tadpole
Exp. 1				
No. 8 (egg + sperm)		84	14	2
No. 9 (egg + sperm)		85	12	3
No. 8 + No. 9 (both are egg + sperm)	+	6	13	81
Exp. 2				
No. 10 (egg + sperm)		96	0	4
No. 11 (egg + sperm)		89	3	8
No. 10 (egg) + No. 11 (sperm)	−	8	5	87
No. 11 (egg) + No. 10 (sperm)	−	23	0	77
Exp. 3				
No. 13 (egg + sperm)		81	17	2
No. 14 (egg + sperm)		86	12	2
No. 16 (egg + sperm)		89	7	4
No. 13 (egg) + No. 16 (sperm)	−	18	15	67
No. 16 (egg) + No. 13 (sperm)	−	11	29	60
No. 14 (egg) + No. 16 (sperm)	+	2	8	90
No. 16 (egg) + No. 14 (sperm)	+	2	12	86
No. 13 (egg) + No. 14 (sperm)	−	8	12	80
No. 14 (egg) + No. 13 (sperm)	−	9	21	70

ent paper reveal another function of coelomic cells from the solitary varieties of ascidians, namely the recognition and destruction of xenogeneic and allogeneic cells via "contact reactions." The reaction is triggered when coelomic cells from two different animals come into contact, though there are exceptional situations in which two allogeneic cells do not react, as discussed further below. The reaction results in almost simultaneous lysis of both cells. Thus, the reaction is bidirectional and ends in a "forced suicide" for both parties. Unlike conventional cell-mediated cytotoxicity of higher vertebrates, an effector-target cell relationship does not seem to hold between two cells in a contact reaction. At least in some cases, diffusible material released by lysis of the first cell may lead to lysis of the second cell and of a nearby "bystander." Nevertheless, the inference is that lysis of the first cell is directly effected by interactions at the membrane level. The absence of autologus contact reactions under a variety of conditions implies involvement of a self-nonself recognition system in this reaction. The effector mechanism, as noted above, may include both cellular and diffusible mediators. A number of morphologically distinct cell types are capable of performing this reaction. However, nothing is known about interrelationships among these cell types, or how they are related to immunocompetent cells of higher vertebrates. Contact reactions were initially found in mixed cultures of xenogeneic or allogeneic coelomic cells and in fact most of the experiments reported here were carried out *in vitro*. However, similar reactions presumably take place in

intact animals if foreign cells have been accidentally introduced into their bodies; inoculation of coelomic cells from one animal into the mantle-test interspace of another animal resulted in lysis of donor cells following formation of cell aggregates between the donor- and host-derived cells.

As already noted, this cellular reaction occurs between allogeneic as well as xenogeneic cells. The sequence of events, the consequence, and the type of cells involved were the same whether the reaction took place in xenogeneic or allogeneic combinations. Allogeneic contact reactions, however, could be observed in most but not all combinations of individuals. A checkerboard-type experiment of 16 *H. roretzi* Type A individuals afforded 120 different reciprocal combinations. Of these, 14 combinations or roughly 12% did not evoke contact reactions (Table I). When alloreactivity of each of the 16 animals was compared, only two individuals had identical patterns and each of the rest of the panel displayed a unique pattern. In addition, three individuals out of 16 (19%) had alloreactivity with all the remaining individuals. These results cannot be explained by applying the familiar rules of transplantation genetics established for higher vertebrates. Absence of alloreactivity between two individuals of the mouse, for instance, indicates lack of disparity in their histocompatibility antigens. Accordingly, these two mice will react exactly the same way against histocompatibility antigens of a third mouse. In the ascidians, however, two histocompatible individuals more often than not differ in their reactivity with a third individual of the same species. For example, animals Nos. 2 and 12 do not react with each other, yet animal No. 6 reacted with No. 2 and not with No. 12, indicating that the former two individuals could not be identical (Table I). Obviously, therefore, alloreactivity in ascidians is governed by a rule different from the one applicable to higher vertebrates. The exact nature of genetic control for alloreactivity of *H. roretzi* must be elucidated employing larger panels of randomly collected individuals.

Most ascidians are hermaphrodites with varying degrees of self-sterility: Normally, fertilization occurs only between gametes from different individuals. In the solitary ascidian, *Ciona intestinalis*, this self-sterility is due to a blockade at the egg membrane (chorion) which prevents penetration of autologous spermatozoa into the egg (Morgan, 1923, 1943). Removal of the membrane by mechanical means makes such eggs fertilizable not only by autologous spermatozoa (Morgan, 1923) but even by spermatozoa from species of other genera, leading in the latter case to the development of interspecific hybrids at high frequencies (Minganti, 1948). It is in this regard that the positive correlation between alloreactivity and fertilizability in the colonial ascidian *B. primigenus* is particularly intriguing, since it raises the possibility that a single histocompatibility locus may control not only the alloreactivity but also other cell-to-cell interactions (Oka and Watanabe, 1957, 1967; Oka, 1970). Rather unexpectedly, however, the results of similar experiments with the solitary *H. roretzi* showed no apparent relationship between allogeneic reactivity and fertilization (Table II). This was not due to indiscriminate fertilization, because self-fertilization frequencies were consistently low in every individual tested. Whatever the exact differences in the mechanism of genetic control of fertilization may be (*e.g.*, germ-line versus somatic origin of egg membrane, surface recognition mechanism, etc.), the difference in the restriction for fertilization strongly suggests that the genetic structures of the populations of these two species are dissimilar.

Studies of self-nonself recognition in invertebrates by means of xenograft or allograft rejections have been hampered by the long observation periods normally

required for end-point determination. In contrast, xenogeneic or allogeneic contact reactions are complete within a few minutes. Moreover, there is no need to sacrifice the donors to remove coelomic cells. Although it remains to be seen if analogous reactions obtain in animal groups further down the phylogenetic tree, the *in vitro* contact reactions should prove to be an excellent model for genetic and biological studies of primitive self-nonself recognition at the cellular level.

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Summary

Coelomic cells from solitary ascidians exhibit a nonphagocytic cellular reaction when they are mixed *in vitro* with such cells from different species or another individual of the same species. The reaction is triggered by direct contact between reactive cells and hence was denoted "contact reaction." The contact reaction is reciprocal and results in mutual cell deaths or a state of prolonged incapacitation of both cells involved. Coelomic cells of several morphological types are capable of carrying out the reaction. Similar reactions were also observed *in vivo* when foreign cells were deliberately introduced into intact animals.

Individuals from a single variant type of *Halocynthia roretzi* (Drasche) are mutually reactive when their cells are brought into contact, but not in every combination of individuals. The patterns of positive and negative contact reactivity among such allogeneic individuals do not follow conventional rules of transplantation genetics established for the vertebrates. These reactions seem to represent a cellular counterpart of non-fusion reactions in the colonial ascidians. However, unlike the colonial ascidians, in which fertilization between gametes from two individual colonies correlates with positive alloreactivity between them, that of solitary *H. roretzi* apparently takes place between any two individuals regardless of their mutual alloreactivity.

The *in vitro* contact reaction is complete within a few minutes. Since coelomic cells can be tapped without sacrificing the donor animals, the *in vitro* contact reaction promises to be an excellent model for further studies of primitive self-nonself recognition at the cellular level.

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